

APPENDIX B
PENDING CLAIMS SUBJECT TO EXAMINATION

1 1. (Twice amended) A method of counting a single copy of a target
2 species immobilized on a substrate, said method comprising:

3 (i) detecting a single copy of said target species by detecting an optical
4 characteristic of a first quantum dot and a second quantum dot attached to said single copy,
5 wherein said single copy is bound to an affinity moiety for said target species immobilized
6 on said substrate, and further wherein said first quantum dot is distinguishable from said
7 second quantum dot.

1 2. (Once amended) The method according to claim 1, wherein said first
2 quantum dot and said second quantum dot are attached to said target species prior to binding
3 said target species to said affinity moiety.

1 3. (Once amended) The method according to claim 1, wherein said first
2 quantum dot and said second quantum dot are attached to said target species after binding
3 said target species to said affinity moiety.

1 5. (Once amended) The method according to claim 1, wherein binding of
2 said target species to said affinity moiety forms a target species-affinity moiety complex that
3 is detected by fluorescence from both said first quantum dot and said second quantum dot
4 attached to said target species-affinity moiety complex.

1 6. (Once amended) The method according to claim 1, wherein said first
2 quantum dot and said second quantum dot are distinguishable by a characteristic which is a
3 member selected from the group consisting of fluorescence spectrum, fluorescence emission,
4 fluorescence excitation spectrum, ultraviolet light absorbance, visible light absorbance,
5 fluorescence quantum yield, fluorescence lifetime, light scattering and combinations thereof.

1 7. (Once amended) The method according to claim 1, wherein said first
2 quantum dot and said second quantum dot are visually distinguishable as a first color and a
3 second color, respectively.

1 8. The method according to claim 7, wherein said first color and said
2 second color combine to form a visually or electronically distinguishable color different from
3 both said first color and said second color.

1 9. The method according to claim 1, wherein said target species has n
2 quantum dots attached thereto, wherein each of said n quantum dots is distinguishable from
3 each other, and n is an integer from 3 to 10.

1 10. (Twice amended) The method according to claim 1, wherein said first
2 quantum dot and said second quantum dot are attached to a targeting moiety for said target
3 species, said targeting moiety being a member selected from the group consisting of
4 antibodies, aptamers, proteins, streptavidin, nucleic acids and biotin.

1 11. The method according to claim 1, wherein said affinity moiety is
2 labeled with a quantum dot.

1 12. The method according to claim 1, wherein said target species is a
2 member selected from the group consisting of organisms, biomolecules and bioactive
3 molecules.

1 13. The method according to claim 1, wherein said affinity moiety is a
2 member selected from the group consisting of organisms, biomolecules and bioactive
3 molecules.

1 14. The method according to claim 1, wherein said substrate has bound
2 thereto a second affinity moiety.

1 15. The method according to claim 14, wherein said first affinity moiety
2 and said second affinity moiety are different affinity moieties.

1 16. The method according to claim 1, wherein said substrate has bound
2 thereto m affinity moieties; and m is an integer from 1 to 10,000.

1 17. The method according to claim 16, wherein each of said m affinity
2 moieties is a different affinity moiety.

1 18. The method according to claim 16, wherein said m affinity moieties
2 are ordered in an array format.

1 19. The method according to claim 1, wherein said substrate further
2 comprises an alignment moiety providing a reference point on said substrate for the detection
3 of a target-affinity moiety complex formed between said target and said affinity moiety,
4 wherein said target-affinity moiety complex is distributed upon said substrate in a random
5 manner, said alignment moiety comprising a fluorescent label, which does not interact with
6 said target species or said affinity moiety.

1 20. The method according to claim 19, wherein said alignment moiety
2 comprises a quantum dot.

1 21. The method according to claim 19, wherein said alignment moiety is
2 distinguishable from each quantum dot attached to said target species.

1 22. (Twice amended) The method according to claim 19, wherein said
2 alignment moiety identifies the position of one or more target moiety-affinity complexes.

1 23. The method according to claim 1, wherein said substrate is
2 manufactured from a low fluorescence optical material configured as a member selected from
3 the group consisting of a microtiter plate, a glass slide, a microscope slide cover slip, a
4 capillary, a flow cell, a bead and combinations thereof.

1 24. The method according to claim 1, further comprising, counting each
2 detected quantum dot per unit area of said substrate, producing substrate quantum dot data;
3 and comparing said substrate quantum dot data with standard quantum dot quantity data

4 acquired from a standard of said quantum dot having a known concentration, thereby
5 quantifying said target species immobilized on said substrate.

1 25. (Once amended) A computer-readable medium encoded with a data set
2 comprising data acquired by the method of claim 1.

1 28. (Once amended) A computer-readable medium encoded with a
2 database comprising two or more data sets according to claim 25, wherein said database is in
3 a searchable format.

1 29. (Twice amended) A method of counting a single copy of a target
2 species in solution, said method comprising
3 (i) detecting a single copy of said target species by detecting essentially
4 simultaneously an optical characteristic of a first quantum dot of a first color attached to said
5 single copy and a second quantum dot of a second color attached to said single copy, wherein
6 said first color and said second color are distinguishably different colors, thereby counting
7 said single copy.

1 30. (Twice amended) A method of counting a single copy of a target
2 species immobilized on a substrate, which species is a member of a population of target
3 species immobilized on said substrate with spacing between each member of said population,
4 said method comprising:

5 (i) detecting a single copy of said target species by detecting an optical
6 characteristic of a first quantum dot and a second quantum dot attached to said single copy,
7 wherein said single copy is bound to an affinity moiety for said target species immobilized
8 on said substrate, wherein said first quantum dot is distinguishable from said second quantum
9 dot, and further wherein said detecting is performed with a detecting means having a
10 resolution that is higher than said spacing between each member of said population, thereby
11 counting said single copy.

1 31. (Twice amended) A method of counting a single copy of a target
2 species immobilized on a substrate, which species is a member of a population of target
3 species immobilized on said substrate, said method comprising:

4 (i) detecting a single copy of said target species by detecting an optical
5 characteristic of a quantum dot attached to said single copy, wherein said first quantum dot is
6 distinguishable from said second quantum dot, and further wherein said single copy is bound
7 to an affinity moiety for said target species immobilized on said substrate forming a target-
8 affinity moiety complex, and said detecting is performed with a detecting means having a
9 resolution limited region of interest whereby, less than one target-affinity moiety complex is
10 present within each resolution limited region of interest, thereby counting said single copy.

1 32. (Twice amended) A method of counting a single copy of a first target
2 species immobilized on a substrate, which species is a member of a population of target
3 species immobilized on said substrate, said method comprising:

4 (a) defining a first region of interest of said substrate; and

5 (b) probing said first region of interest for an optical characteristic of a first
6 quantum dot and a second quantum dot attached to said single copy of said first target species
7 bound to an affinity moiety for said first target species immobilized on said substrate,
8 wherein said first quantum dot is distinguishable from said second quantum dot, thereby
9 counting said first target species.

1 33. (Twice amended) The method according to claim 32, further
2 comprising counting a single copy of a second target species immobilized to said substrate,
3 said method comprising:

4 (c) defining a second region of interest of said substrate; and

5 (d) probing said second region of interest for an optical characteristic of a
6 third quantum dot and a fourth quantum dot attached to said single copy of said second target
7 species bound to an affinity moiety for said second target species immobilized on said
8 substrate, wherein said third quantum dot is distinguishable from said fourth quantum dot,
9 thereby counting said second target species.

1 34. The method according to claim 33, wherein said first region of interest
2 and said second region of interest are the same region of interest.

1 35. The method according to claim 32, wherein said probing is by a
2 method selected from the group consisting of microscopy, confocal fluorescence microscopy
3 and two-dimensional imaging with a CCD camera.

1 36. The method according to claim 32, wherein said first target species
2 and said second target species are different species.

1 37. (Once amended) A method for counting multiple target species
2 immobilized on a substrate, which species are members of a population of target species
3 immobilized on said substrate, said method comprising:

4 (a) defining multiple regions of interest on said substrate; and

5 (b) probing said multiple regions of interest for an optical characteristic of a
6 first quantum dot and a second quantum dot attached to a single copy of said target species
7 bound to an affinity moiety for said target species immobilized within a region of interest of
8 said substrate, thereby counting multiple target species.

1 38. (Once amended) A method for determining whether a target species
2 within a region of interest on a substrate is quantifiable by a technique selected from the
3 group consisting of single target counting and ensemble counting, said method comprising:

4 (a) probing said region of interest to determine target species density within
5 said region of interest by detecting fluorescence emitted by a quantum dot attached to one or
6 more molecules of said target species bound to an affinity moiety for said target species
7 immobilized on said substrate;

8 (b) comparing said density to a predetermined density cutoff value above
9 which ensemble counting is used and below which single target counting is used, thereby
10 determining whether said target species is quantifiable by target counting or ensemble
11 counting.

1 39. The method according to claim 38, wherein said substrate comprises a
2 first region in which ensemble counting is used and a second region in which single target
3 counting is used.

1 40. (New) The method according to claim 1, wherein said optical
2 characteristic is detected by coincidence detection.

1 41. (New) The method according to claim 1, wherein said optical
2 characteristic is fluorescence.

1 42. (New) The method according to claim 29, wherein said optical
2 characteristic is fluorescence.

1 43. (New) The method according to claim 31, wherein said optical
2 characteristic is fluorescence.

1 44. (New) The method according to claim 32, wherein said optical
2 characteristic is fluorescence.

1 45. (New) The method according to claim 33, wherein said optical
2 characteristic is fluorescence.

1 46. (New) The method according to claim 37, wherein said optical
2 characteristic is fluorescence.

1 47. (New) The method according to claim 1, further comprising
2 (ii) resolving said optical characteristic of said first quantum dot and said
3 second quantum dot attached to said single copy from an optical characteristic arising from a
4 quantum dot not attached to said single copy.

1 48. (New) The method according to claim 29, further comprising

2 (ii) resolving said optical characteristic of said first quantum dot and said
3 second quantum dot attached to said single copy from an optical characteristic arising from a
4 quantum dot not attached to said single copy.

1 49. (New) The method according to claim 32, further wherein said probing
2 resolves said optical characteristic of said first quantum dot and said second quantum dot
3 from an optical characteristic of other members of said population of target species
4 immobilized on said substrate.

1 50. (New) The method according to claim 33, further wherein said probing
2 resolves said optical characteristic of said third quantum dot and said fourth quantum dot
3 from an optical characteristic of other members of said population of target species
4 immobilized on said substrate.

1 51. (New) The method according to claim 37, wherein said probing
2 resolves the optical characteristic of said first quantum dot and said second quantum dot from
3 other members of said population and from each other.